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STRUCTURE OF THE POLYMERS**

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ABSTRACT

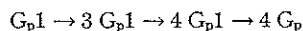
The soluble poly- β -D-glucan from oats has been subjected to degradation by two different types of enzymes. "Cellulase" converts the polysaccharide to a trisaccharide, 4-O- β -D-laminaribiosyl-D-glucose, and two tetrasaccharides, 3'-O- β -D-cellobiosyl-D-cellobiose and 4'-O- β -D-laminaribiosyl-D-cellobiose.* Degradation by the second enzyme, "laminarinase", produces a trisaccharide, 3-O- β -D-cellobiosyl-D-glucose, and a tetrasaccharide, 3-O- β -D-cellobiosyl-D-glucose.† These products, which account for 75–85% of the polymer in each experiment, have been characterized by chemical methods. The data show that the glucan is composed almost entirely of two types of structural sequences: one is a tetrameric unit in which a single β -(1 \rightarrow 3) linkage alternates with two β -(1 \rightarrow 4) linkages, and the other, a pentameric unit in which a single β -(1 \rightarrow 3) linkage alternates with three consecutive β -(1 \rightarrow 4) linkages.

The soluble poly- β -D-glucan from barley has been shown by enzymolysis with the "cellulase" to be closely related in detailed structure to the oat polymer.

Steric aspects of the enzymic degradations are discussed.

The grains of oats and barley contain water-soluble polysaccharides which are composed of β -D-glucopyranosyl units (1, 2, 3, 4, 5). In barley, the polyglucan appears to be closely associated with the malting process, during which there occurs a large increase in the proportion of the water-soluble polymer. Results from several investigations (6, 7, 8, 9) have shown that these polysaccharides are linear and that the monomeric units are joined by either (1 \rightarrow 3) or (1 \rightarrow 4) β -D-glycosidic linkages. An examination by Peat, Whelan, and Roberts (9) of the products formed by partial acid hydrolysis of oat glucan, has demonstrated clearly that both types of linkage occur together in the same chain, and that a major proportion of the polymer may be represented by a tetrameric repeating unit (I) in which two (1 \rightarrow 4) linkages and a (1 \rightarrow 3) linkage alternate. Lichenin, a poly- β -D-glucan from Iceland moss, was found also by this approach to be constituted in a closely similar fashion (9).

In the current study, the β -D-glucans from oats and barley have been degraded selectively with two different enzymic preparations, a "cellulase" and a "laminarinase". From the identity of the products formed detailed information about the structure of the polysaccharides has been obtained. The basic type of formula (I) advanced by Peat, Whelan, and Roberts has been confirmed for the oat glucan, and shown to apply also for barley glucan, and additional data about the constitution of these polymers provided.



I

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*For the sake of brevity, trivial names are used where possible in describing the oligosaccharides reported in this paper. More formal descriptions of the trisaccharide and two tetrasaccharides, respectively, are: O- β -D-glucopyranosyl-(1 \rightarrow 3)-O- β -D-glucopyranosyl-(1 \rightarrow 4)-D-glucose; O- β -D-glucopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl-(1 \rightarrow 3)-O- β -D-glucopyranosyl-(1 \rightarrow 4)-D-glucose; and O- β -D-glucopyranosyl-(1 \rightarrow 3)-O- β -D-glucopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl-(1 \rightarrow 4)-D-glucose.

†O- β -D-Glucopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl-(1 \rightarrow 3)-D-glucose and O- β -D-glucopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl-(1 \rightarrow 3)-D-glucose, respectively.

Enzymic Degradation by Cellulase

The glucans were found to be hydrolyzed rapidly and extensively by a cellulase isolated from *Streptomyces* sp. QM B814. This enzyme preparation hydrolyzes cellulose in high yield to cellobiose and cellotriose (10). Under conditions expected to minimize transglycosylation side-reactions, the products formed from both glucans were mainly one tri- and two tetra-saccharides, and only minor or trace proportions of higher oligosaccharides, disaccharides, and glucose were detected. The products (Table I) were isolated by preparative paper chromatography.

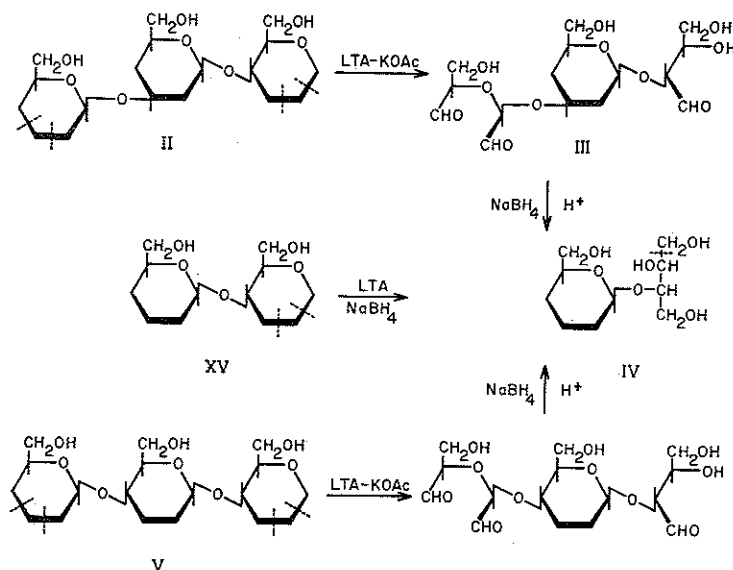
TABLE I
Yields of products from enzymolysis of poly- β -D-glucans*

Fraction	Cellulase		Laminarinase
	Barley glucan	Oat glucan	Oat glucan
Trisaccharide	628	638	520
Tetrasaccharides	303	337	302
Disaccharide	68	75	61
Glucose	9	8	81
Unidentified†	93	67	—

*Milligrams of crude product/1.0 g. polyanhydroglucose. Figures in parentheses are moles trisaccharide : moles tetrasaccharide.

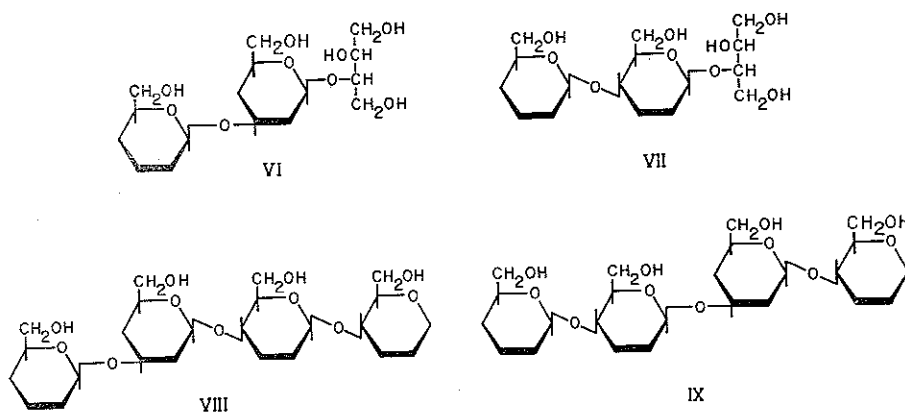
†Components remaining close to the origin on paper chromatograms.

The major product obtained from each glucan (yield, about 60%) was an amorphous trisaccharide, which was purified by conversion to a crystalline acetate. The physical constants of the derived acetate corresponded to those reported for 4-*O*- β -D-laminaribiosyl-D-glucose undecaacetate (9), and the free trisaccharide was found to possess this structure (II)* in the following way. On treatment with lead tetraacetate in the presence of potassium acetate the compound produced formic acid at the same rate as cellotriose (11), and consumed 4 moles of oxidant, consistent with the formulation of a



*All hexopyranosyl units represented by these formulae possess the D-glucopyranose configuration.

4-substituted reducing end-unit, and the production of III. The oxidation product was reduced with sodium borohydride (12), and selectively hydrolyzed with acid under the conditions recommended by Smith and co-workers (13), yielding 2-*O*- β -D-glucopyranosyl-D-erythritol (IV) (14). Hence the central unit of the original trisaccharide was attached to the reducing end-unit by a β -(1 \rightarrow 4) linkage. The validity of this degradation procedure was checked with celotriose (V), which also yielded IV since the central unit in this trisaccharide is resistant to lead tetraacetate oxidation under the conditions used (11). In the absence of potassium acetate, a relatively slow uptake of about 1.7 moles of lead tetraacetate was observed with II, characteristic of a 4-substituted D-glucose (14), and reduction of the product afforded 2-*O*- β -D-laminaribiosyl-D-erythritol (VI). Presence of the β -(1 \rightarrow 3) linkage in II was confirmed by partial hydrolysis, which afforded laminaribiose, characterized as the octaacetate.



The amorphous tetrasaccharide fraction obtained from the oat and barley glucans accounted for about 30% of the original polysaccharides. Oxidation of each fraction with lead tetraacetate - potassium acetate, followed by reduction of the oxidized tetramer with sodium borohydride and selective acid hydrolysis (13), yielded two products in a ratio of approximately 3 to 2. The major product was 2-*O*- β -D-cellobiosyl-D-erythritol (VII) and the other, 2-*O*- β -D-laminaribiosyl-D-erythritol (VI). Isolation of these two triitols showed that the tetrasaccharide fractions contained two components (not separable by paper chromatography), one containing a central β -(1 \rightarrow 4) linkage and the other a central β -(1 \rightarrow 3) linkage, and both having a β -(1 \rightarrow 4) linkage adjacent to the reducing end-unit. One of these components was isolated by fractional crystallization,* and when degraded according to the reaction sequence described above afforded VII. Also, on reduction followed by partial acid hydrolysis, the crystalline tetrasaccharide gave laminaribiose, cellobiose, and trisaccharide (II). These properties therefore characterized the compound as 4'-*O*- β -D-laminaribiosyl-D-cellobiose (VIII). The second tetrasaccharide component was amorphous. However, it was shown to be 3'-*O*- β -D-cellobiosyl-D-cellobiose (IX), since it yielded VI on selective degradation (as above), and after reduction followed by partial hydrolysis it afforded cellobiose, laminaribiose, and trisaccharide (X).

A detailed structure for the major proportion (about 85%) of the oat and barley β -D-glucans may clearly be formulated from the identity of the three oligosaccharides obtained by enzymolysis. Since the molar ratio of trisaccharide to tetrasaccharide formed

*Two modifications of this tetrasaccharide were obtained, the α -anomer and a dihydrate of the β -anomer.

is about 2.5 to 1, and since (on the average) about seven glucose units are linked by (1 \rightarrow 4) bonds and three by (1 \rightarrow 3) bonds, a structural relationship between polymer and products may be depicted as in Fig. 1.* According to this scheme, the polymers

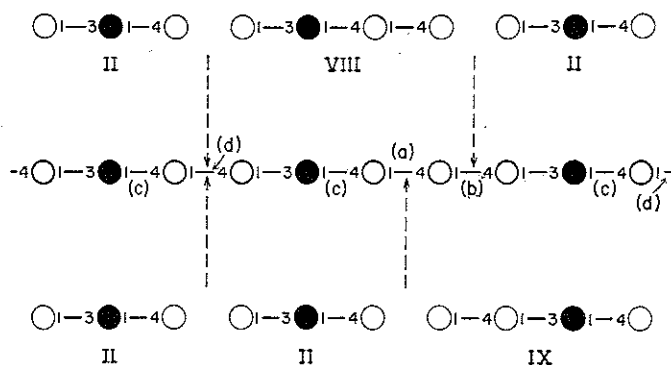


FIG. 1. Structural relationship between oat and barley poly- β -D-glucan (central row of circles) and oligosaccharides produced by the action of "cellulase". All circles represent β -D-glucopyranosyl units; those units which are bonded glycosidically through positions-1 and -3 are in black, for contrast.

contain two types of repeating units represented by (a) a tetrameric unit (I, page 2094) in which a single β -(1 \rightarrow 3) linkage alternates with two consecutive β -(1 \rightarrow 4) linkages (9), and (b) a pentameric unit in which a single β -(1 \rightarrow 3) linkage alternates with three consecutive β -(1 \rightarrow 4) linkages. The (1 \rightarrow 3) bonds occur solely in isolated positions, but there can be few, if any, isolated (1 \rightarrow 4) bonds in the molecule.[†] Rather, the (1 \rightarrow 4) linkages are found most frequently in pairs and less often as a sequence of three, but the available evidence suggests that there are never more than three (1 \rightarrow 4) linkages in consecutive order along the polymer chain.

Formation by the cellulase of the two tetrasaccharides VIII and IX appears to involve cleavage of bond (b) or (a), respectively (Fig. 1), 2 moles of trisaccharide II being formed in either instance. Since more of VIII than IX is produced, bond (b) is the more easily cleaved although the difference is rather small (ratio of 3.5 to 2). However, it may be noted that bond (c) is *not* cleaved by the enzyme, although it is also of the β -(1 \rightarrow 4) type usually associated with cellulase action. As noted elsewhere (15), the D-glucosyl unit to the left of bond (c) is substituted at position-3, rather than at position-4, making for a "(\rightarrow 3)- β -(1 \rightarrow 4)" relationship. By contrast, linkages (a), (b), and (d), which possess a "(\rightarrow 4)- β -(1 \rightarrow 4)" arrangement, all are cleaved by the cellulase.

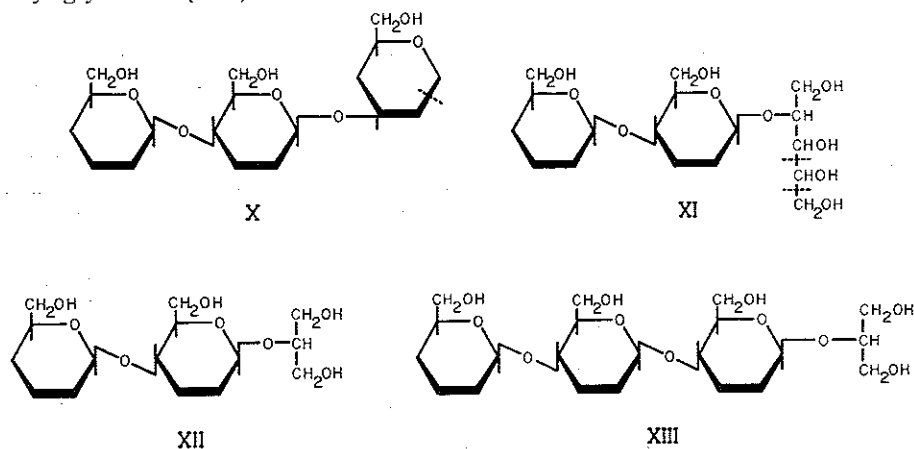
Enzymic Degradation by Laminarinase

A second type of enzyme, classified as a "laminarinase" by Reese and Mandels (16) since it shows high specificity for laminarin, was also found capable of rapidly degrading the β -D-glucans. This glycosidase is an endo-enzyme produced by *Rhizopus arrhizus* QM 1032. Acting on laminarin, it yields laminaribiose and laminaritriose as the dominant products (16). With the oat polymer, a tri- and tetra-saccharide were detected in the hydrolyzate together with a small proportion of laminaribiose. The major product, which

*For simplicity, the ratio of tri- to tetra-saccharide in Fig. 1 is 2 to 1. However, closer agreement is obtained with the ratio found experimentally by considering a larger repeating structure (e.g., one containing 20-25 units). The molecular weight of the polysaccharides was not estimated, but the low yield of formic acid produced during periodate oxidation of the materials (experimental section) indicated a D.P. value of not less than 50-100 units.

[†]Oligosaccharides having D.P. greater than 4, which were formed in low yield (Table I), were not examined and may represent minor structural sequences not accommodated by Fig. 1.

accounted for about 50% of the glucan, was an amorphous trisaccharide, characterized as the derived acetate. This trisaccharide was shown to be 3-*O*- β -D-cellobiosyl-D-glucose (X)* by the following reactions. It rapidly consumed 1 mole of lead tetraacetate per mole and yielded an equivalent of pentose showing that the reducing end-unit was substituted at position-3 (14). In agreement with this assignment the compound migrated on an electrophoretogram (18) at a rate close to that of laminaribiose. The oxidized trisaccharide was reduced to the corresponding glycitol (XI, not isolated) which was treated in turn with 2 moles of lead tetraacetate, followed by a second reduction. This degradation procedure had been used earlier to convert a 3-linked hexose disaccharide to a 2-*O*-glycosyl-glyceritol (19). Accordingly, the final product obtained was 2-*O*- β -D-cellobiosyl-glyceritol (XII).



The tetrasaccharide, which accounted for 30% of the polymer, was crystalline. Its behavior on oxidation with lead tetraacetate and ionophoresis paralleled that of the trisaccharide (X) (above), showing that this compound also possesses a 3-substituted reducing end-unit. By selectively degrading the reducing end-unit to a 2-substituted glyceritol unit (as for X to XII), 2-*O*- β -D-cellobiosyl-glyceritol (XIII) was obtained. Accordingly, the tetrasaccharide produced by laminarinase action is 3-*O*- β -D-cellobiosyl-D-glucose (XIV, Fig. 2).

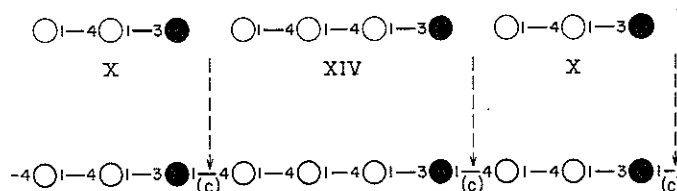


FIG. 2. Structural relationship between oat poly- β -D-glucan (lower row of circles) and oligosaccharides produced by the action of "laminarinase". All circles represent β -D-glucopyranosyl units; those units which are bonded glycosidically through positions-1 and -3 are in black, for contrast.

Together, the tri- and tetra-saccharide account for about 80% of the original oat β -D-glucan and are produced in a molar ratio of about 2.3 to 1. As shown in Fig. 2, formation of these products can readily be accommodated by the polymeric structure

*Two sets of physical constants have been reported for the undecaacetate of this compound; *m.p.* 110° C, $[\alpha]_D$ -8° (9) and *m.p.* 183° C, $[\alpha]_D$ -22° (17). (The acetate obtained in the current study has the same properties as recorded in reference 9.) Consequently, a confirmation of the structure of X appeared necessary, and is reported herein.

already depicted in Fig. 1.* With laminarinase, cleavage was specific for linkage (c), i.e., one adjacent to a β -D-glucosyl unit *substituted at position-3*, rather than the β -(1 \rightarrow 3) bond itself (15). Hence, in contrast to the action of cellulase, only one tetrasaccharide was obtained. These results, therefore, fully complement those obtained by enzymolysis of the β -D-glucans with cellulase, and show clearly that the major proportion of the polysaccharides is constituted as depicted in Figs. 1 and 2. It is possible that the glucans comprise a mixture of molecules, some of which contain only the tetrameric repeating unit and others only the pentameric unit. However, formation of the two tetrasaccharides by the "cellulase" is accommodated most readily by assuming that both types of repeating units occur interspersed within the same molecule. Of possible significance in this connection is the fact that fractional precipitation of barley glucan yielded several fractions, each of which was hydrolyzed to the same degree by cellulase and gave the same mixture of products.

EXPERIMENTAL

Paper chromatography was carried out with ethyl acetate:pyridine:water (10:4:3) as solvent (20); for preparative chromatography Whatman 3MM paper was used. Spray reagents used were aniline oxalate (21) and ammoniacal silver nitrate (22). R_G represents the rate of travel of a compound on a chromatogram relative to the rate for glucose.

Paper electrophoresis (18) was carried out using borate buffer (pH 9.2 (23)) at 10 volts per centimeter.

Copper-reducing power was measured by the method of Nelson (24).

Solutions were evaporated at 40–50° C, and optical rotations were measured at approximately 25° C. Melting points are corrected.

Oat flour was milled from Fortune oats grown at the University of Saskatchewan, and barley flour from husky six-rowed barley grown in the Saskatoon area.

Isolation of Polyglucans

A combination of the procedures described by Morris (2) and by Meredith, Watts, and Anderson (5) was used. Oat or barley flour (3 kg), previously heat-inactivated with boiling 80% ethanol, was extracted with water (24 liters) for 3 hours in the presence of papain (6 g). The digest was centrifuged and ammonium sulphate (2200 g) was added to the supernatant solution causing flocculation of the polyglucan. The latter was collected, washed with 50% ethanol until sulphate-free, then with acetone, and air-dried. The barley glucan (19.0 g) was a white powder, $[\alpha]_D^{22} -13.1^\circ$ (c, 0.9, H₂O). Found: anhydroglucose (copper-reducing power after hydrolysis), 89.0%; N, 0.37%; ash, 0.81%; moisture, 12.4%. The oat glucan (26.0 g) was a white fibrous material, $[\alpha]_D^{27} -8.3^\circ$ (c, 0.9, H₂O). Found: anhydroglucose, 86.9%; N, 0.36%; ash, 0.17%; moisture, 10.2%.

Periodate Oxidation of Polyglucans

By oxidation of the polyglucans with 2 molar equivalents of sodium metaperiodate at 20° C in the dark, the following results were obtained:

Periodate consumed by barley glucan (moles/mole (hours)): 0.72 (20), 0.73 (45), 0.79 (95), 0.81 (141). Formic acid production (moles/mole) at 20 and 141 hours was 0.005 and 0.055, respectively, giving a corrected value of about 0.75 mole periodate consumed per anhydroglucose unit at 141 hours.

Closely similar data were obtained with the oat glucan. Periodate consumption (moles/

*See footnote, p. 2097.

mole (hours)): 0.65 (23), 0.76 (47), 0.77 (102), 0.80 (143). At 47 hours the yield of formic acid was 0.016 mole/mole.

Degradation of Barley Glucan Fractions with Cellulase

Ethanol was added dropwise to a vigorously stirred barley glucan solution (500 mg, 22.5 ml). Precipitates obtained (weights in parentheses) between zero and 17% ethanol (115 mg), 17–23% (276 mg), 23–31% (57 mg), 31–47% (21 mg), and 47–64% (5 mg) were collected and dried. The first four fractions (18 mg) were incubated in water (1.7 ml) at 48° C and pH 6 with cellulase (6 mg). No difference was observed between fractions in rate of hydrolysis (copper-reducing power). In addition, when the reducing power became constant (at glucose equivalent of 25%, 2 hours), paper chromatographic examination of the digests showed the presence in each of two main products, corresponding to a tri- and tetra-saccharide, respectively (R_G 0.45 and 0.22). Of the three minor products detected, two were indistinguishable from glucose and cellobiose, and the third had R_G 0.10.

Further experiments with the unfractionated glucan showed that the glucan:cellulase ratio could be increased to 8:1 with little effect on the degree of hydrolysis attained and the type of products formed.

Degradation of Oat and Barley Glucans with Cellulase

Oat or barley glucan (1.0 g) in water (100 ml) was incubated at 48° C with cellulase (0.12 g) for 5 hours. The enzyme was then inactivated by heating the digest for 10 minutes on the boiling-water bath, the digest was filtered, concentrated, and the syrup obtained was chromatographed on Whatman 3 MM paper sheets. After elution from the papers with water, the sugars were purified by use of mixed-bed ion-exchange resins. Yields of individual sugars are given in Table I.

Products from the Cellulase Degradation of Barley Glucan

4-O- β -D-Laminaribiosyl-D-glucose (II)

The trisaccharide fraction was chromatographically homogeneous containing neither laminaritriose nor cellotriose, $[\alpha]_D +11.6^\circ$ (c , 1.3, H_2O). Partial hydrolysis of the material yielded cellobiose, laminaribiose, and glucose (paper chromatogram). On treatment with lead tetraacetate the rate of uptake of oxidant was closely similar to that of cellotriose and cellobiose (11, 14), and in the presence of potassium acetate the lead tetraacetate oxidation characteristics were those of a hexopyranosyl trisaccharide possessing a 4-linked reducing end-unit (11).

4-O- β -D-Laminaribiosyl- β -D-glucose Undecaacetate

A portion (53 mg) of II was heated at 100° C for 2 hours with acetic anhydride containing anhydrous sodium acetate. After evaporation of the acetic anhydride the product was isolated by extraction into chloroform and crystallized from ethanol (weight, 75 mg), m.p. 120–122° C and $[\alpha]_D -20.0^\circ$ (c , 1.5, $CHCl_3$) (lit. (9) m.p. 121–123° C and $[\alpha]_D -22.2^\circ$). Calculated for $C_{40}H_{54}O_{27}$: C, 49.69%; H, 5.63%; acetyl, 48.98%. Found: C, 49.55%; H, 5.70%; acetyl, 49.10%.

Deacetylation of the material in the mother liquors, followed by paper chromatography, indicated the presence of II only.

Degradation of II to 2-O- β -D-Glucopyranosyl-D-erythritol

The amorphous material (149 mg) in water (1 ml) was taken up in acetic acid (9 ml) and treated with a solution of lead tetraacetate (660 mg, 5 molar proportions) and potassium acetate (400 mg) in acetic acid (27 ml). After 11 hours the excess tetravalent

lead was reduced with a 10% solution of oxalic acid in acetic acid (0.2 ml), and the solution was treated with excess Amberlite IR-120 and concentrated. By repeated addition of ethanol and toluene to the residue, and evaporation, the last traces of acetic acid were removed. The final residue was dissolved in water (5 ml) and added slowly to a solution of sodium borohydride (150 mg) in ice water (5 ml). After 16 hours, sodium ions were removed with Amberlite IR-120 and borate as the volatile methyl ester, and the reduced product was treated with 0.1 *N* sulphuric acid at room temperature for 16 hours (13). The hydrolyzate was neutralized with Dowex-1 resin and concentrated, and was found by paper chromatographic examination to contain two main components indistinguishable from 2-*O*- β -D-glucosyl-D-erythritol and glycerol. This mixture (105 mg) was fractionated on a column of coconut charcoal (25) (5 g) by gradient elution with aqueous ethanol up to a concentration of 20% ethanol, affording 2-*O*- β -D-glucopyranosyl-D-erythritol (40 mg), m.p. 191–194° C and $[\alpha]_D -15.2^\circ$ (*c*, 1.3, H₂O). The X-ray diffraction pattern of the compound was indistinguishable from that of an authentic sample (14). Calculated for C₁₀H₂₀O₉: C, 42.25%; H, 7.09%. Found: C, 42.42%; H, 7.39%.

Degradation of II to Laminaribiose

A portion (800 mg) of II was hydrolyzed with 0.33 *N* sulphuric acid (30 ml) for 1 hour at 100° C, the hydrolyzate was neutralized, concentrated, and fractionated on a cellulose column using butanol saturated with water as solvent. Laminaribiose (117 mg) was isolated and characterized by conversion to the β -octaacetate, m.p. 163–165° C and $[\alpha]_D -26.2^\circ$ (*c*, 2, CHCl₃), which gave an X-ray powder diagram indistinguishable from that of an authentic specimen. Calculated for C₂₈H₃₈O₁₉: C, 49.56%; H, 5.65%. Found: C, 49.38%; H, 5.78%.

2-O- β -D-Laminaribiosyl-D-erythritol (VI)

4-*O*- β -D-Laminaribiosyl-D-glucose (II) (200 mg) in water (0.5 ml) was taken up in acetic acid (30 ml) and oxidized with lead tetraacetate (320 mg). After 5 hours excess tetravalent lead was reduced and divalent lead precipitated by addition of excess 10% oxalic acid in acetic acid. The product was reduced with sodium borohydride and crystallized from methanol-ethanol, m.p. 164–166° C and $[\alpha]_D -23.8^\circ$ (*c*, 1.5, H₂O). Calculated for C₁₆H₃₀O₁₄: C, 43.05%; H, 6.77%. Found: C, 42.84%; H, 7.08%.

2-O- β -D-Cellobiosyl-D-erythritol (VII) and 2-O- β -D-Laminaribiosyl-D-erythritol (VI) from the Tetrasaccharide Fraction

The tetrasaccharide fraction appeared to be chromatographically homogeneous, $[\alpha]_D +9^\circ$ (*c*, 3.1, H₂O). The copper-reducing power ratio before and after acid hydrolysis was 1:3.9 (requires 4.0).

A portion (229 mg) of the material was oxidized in 90% acetic acid with lead tetraacetate (916 mg, 6 molar proportions) in the presence of potassium acetate (440 mg) for 11.5 hours. By the procedure described above, the oxidation product was recovered, reduced with sodium borohydride, and partially hydrolyzed. Paper chromatographic examination of the hydrolyzate showed the presence of two triitol components which, after separation by preparative paper chromatography, were found to be:

(a) 2-*O*- β -D-Laminaribiosyl-D-erythritol (VI) (29 mg).—Crystallized from methanol-ethanol, it had a melting point of 171–173° C and gave an X-ray powder diagram indistinguishable from that of the authentic material.

(b) 2-*O*- β -D-Cellobiosyl-D-erythritol (VII) (65 mg)—which, after crystallization from aqueous methanol, had a melting point of 209–211° C, $[\alpha]_D -8.3^\circ$ (*c*, 1.2, H₂O), and gave an X-ray powder diagram indistinguishable from that of a specimen prepared from

cellotriase* (27). Calculated for $C_{16}H_{30}O_{14}$: C, 43.05%; H, 6.77%. Found: C, 42.86%; H, 6.84%.

Isolation of these two products showed that the original fraction contained two tetrasaccharide components. The ratio of the tritols, estimated by the anthrone method (26) after elution of the products from paper chromatograms, was 1.8 to 1.

4'-O-β-D-Laminaribiosyl-α-D-cellobiose (VIII)

The tetrasaccharide fraction (243 mg) afforded crystalline material (120 mg) from solution in aqueous methanol, m.p. 221–224° C (decomp.), $[\alpha]_D +12.9^\circ$ (2 minutes) $\rightarrow +10.6$ (45 minutes, constant) (*c*, 3.6, H_2O). Calculated for $C_{24}H_{42}O_{21}$: C, 43.24%; H, 6.35%. Found: C, 43.16%; H, 6.67%.

Degraded by the procedure described in the previous section, the crystalline tetrasaccharide afforded 2-O-β-D-cellobiosyl-D-erythritol (VII), but no laminaribiosyl-erythritol. Reduction of the tetrasaccharide with sodium borohydride, followed by partial acid hydrolysis with 0.33 *N* sulphuric acid at 100° C for 1 hour, yielded glucose, laminaribiose, cellobiose, and 4-O-β-D-laminaribiosyl-D-glucose (II) (paper chromatographic and electrophoretic evidence).

3'-O-β-D-Cellobiosyl-D-cellobiose (IX)

After separation of the crystalline tetrasaccharide (VIII) a second crop of impure VIII was precipitated by addition of ethanol to the mother liquor. Concentration of the supernatant solution afforded an amorphous product (80 mg), $[\alpha]_D +19.8^\circ$ (*c*, 1.6, 90% CH_3COOH). On oxidation with lead tetraacetate–potassium acetate followed by reduction and partial hydrolysis, this material was found chromatographically to account for the production of 2-O-β-D-laminaribiosyl-D-erythritol (VI) from the tetrasaccharide fraction; a minor proportion of the cellobiosyl-erythritol (VII) was also detected. The material was reduced with sodium borohydride, then hydrolyzed with hot 0.33 *N* sulphuric acid for 1 hour, yielding glucose, laminaribiose, cellobiose, and 3-O-β-D-cellobiosyl-D-glucose (X) (paper chromatographic and electrophoretic evidence).

Products from the Cellulase Degradation of Oat Glucan

4-O-β-D-Laminaribiosyl-D-glucose (II)

This product, which was chromatographically homogeneous, was characterized by conversion to the β-undecaacetate (540 mg from 310 mg of free sugar), m.p. 120–122° C and $[\alpha]_D -22.7^\circ$ (*c*, 1.5, $CHCl_3$). Calculated for $C_{40}H_{54}O_{27}$: C, 49.69%; H, 5.63%; acetyl, 48.98%. Found: C, 49.77%; H, 5.55%; acetyl, 48.73%.

Tetrasaccharide Fraction

The amorphous product afforded crystalline material from aqueous methanol, m.p. 223–227° C (decomp.), indistinguishable from VIII by comparison of X-ray diffraction powder diagrams. Calculated for $C_{24}H_{42}O_{21}$: C, 43.24%; H, 6.35%. Found: C, 43.04%; H, 6.38%.

In one experiment, a second crystalline modification of this tetrasaccharide was obtained and found to be a dihydrate of the β-anomer, m.p. 180–181° C and $[\alpha]_D +7^\circ$ (2 minutes) $\rightarrow +10^\circ$ (constant) (*c*, 2, H_2O). Calculated for $C_{24}H_{42}O_{21} \cdot 2H_2O$: C, 41.02%; H, 6.60%. Found: C, 41.05%; H, 6.76%. By seeding, the higher-melting α-form (m.p. 221–223° C) of the tetrasaccharide obtained from barley glucan (above) was converted to this lower-melting modification.

By the degradative procedure described above the original, amorphous, tetrasaccharide

*2-O-β-D-Cellobiosyl-D-erythritol was prepared from cellotriase by oxidation with 1.8 molar proportions of lead tetraacetate followed by reduction with sodium borohydride, as in the conversion of cellobiose to 2-O-β-D-glucopyranosyl-D-erythritol (14).

fraction was shown (chromatographically) to yield both cellobiosyl-erythritol and laminaribiosyl-erythritol in a ratio of 1.7 to 1 (anthrone method (26)).

Enzymic Degradation of Oat Glucan with Laminarinase

Oat glucan (9.3 mg) in water (1.5 ml) was incubated at 40° C with laminarinase (1, 2 or 3 mg). At the two highest levels of enzyme concentration the copper-reducing power was virtually constant in 2 to 3 hours, when the apparent conversion to glucose was about 25%. A substantially slower reaction was observed at the substrate:enzyme ratio of 9:1. Examined by paper chromatography, all three digests were found to contain two major components which corresponded to a tri- and tetra-saccharide, respectively (R_G 0.47 and 0.22). Minor components detected were glucose, laminaribiose, and material having R_G 0.04.

Oat glucan (1.5 g) in water (100 ml) was incubated with laminarinase (0.25 g) for 2.5 hours. The digest was heated on the boiling-water bath for 7 minutes, filtered, and concentrated. The sugar mixture was fractionated by preparative paper chromatography; individual components were eluted and purified with mixed ion-exchange resins. Yields of the products are given in Table I.

3-O- β -D-Cellobiosyl-D-glucose (X)

The amorphous trisaccharide fraction was chromatographically and electrophoretically homogeneous, $[\alpha]_D +12.1^\circ$ (c , 1.7, H_2O). On oxidation with lead tetraacetate in 97% acetic acid an uptake of 0.8 mole of oxidant per mole was found in 15–30 minutes and, in 90% acetic acid and in the presence of potassium acetate, the consumption of lead tetraacetate was 2.9 moles per mole. In both oxidations, approximately 1 mole of pentose (estimated colorimetrically (28)) was produced per mole of trisaccharide, the over-all data being consistent with presence of a 3-linked reducing end D-glucose unit.

Acetylation of the material (225 mg) with hot acetic anhydride–sodium acetate yielded a β -undecaacetate (340 mg), m.p. 104–105° C and $[\alpha]_D -9.7^\circ$ (c , 1.6, $CHCl_3$). Calculated for $C_{40}H_{54}O_{27}$: C, 49.69%; H, 5.63%; acetyl, 48.98%. Found: C, 49.42%; H, 5.69%; acetyl, 49.37%.

2-O- β -D-Cellobiosyl-glyceritol (XII)

Trisaccharide X (264 mg) in water (1.5 ml) was taken up in acetic acid (50 ml) and treated with lead tetraacetate (232 mg, 1 molecular proportion) for 2 hours. Excess oxalic acid was added to precipitate the divalent lead, and the filtrate was concentrated. The residue in water (2 ml) was neutralized with sodium bicarbonate and the solution added dropwise to ice-cold sodium borohydride solution (264 mg, 12 ml). After 16 hours the reaction mixture was worked up, affording a solid product (227 mg), which was treated in 97% acetic acid (24 ml) with lead tetraacetate (424 mg, 2 molar proportions) for 16 hours. A second reduction with sodium borohydride followed, and the final product was crystallized from aqueous ethanol, m.p. 138–141° C and $[\alpha]_D -15.6^\circ$ (c , 2.5, H_2O). The compound gave an X-ray diffraction pattern indistinguishable from that of an authentic sample* (27). Calculated for $C_{15}H_{28}O_{13}$: C, 43.27%; H, 6.78%. Found: C, 43.00%; H, 6.83%.

3-O- β -D-Cellotriosyl- α -D-glucose (XIV)

The tetrasaccharide fraction readily crystallized from solution in hot aqueous ethanol, m.p. 241–245° C (decomp.) and $[\alpha]_D +11.4^\circ$ (5 minutes) $\rightarrow +8.4^\circ$ (3 hours, constant)

* Prepared from 2-O- β -D-cellobiosyl-D-erythritol (VII) by oxidation with 1 molar proportion of lead tetraacetate followed by reduction with sodium borohydride, as in the degradation of 2-O- β -D-glucopyranosyl-D-erythritol to 2-O- β -D-glucopyranosyl-glyceritol (14).

(c , 2.7, H_2O). Calculated for $C_{24}H_{42}O_{21}$: C, 43.24%; H, 6.35%. Found: C, 43.19%; H, 6.37%.

Treated in 97% acetic acid with 3 molar proportions of lead tetraacetate, the tetrasaccharide (20 mg) consumed 0.91, 0.96, and 0.99 mole of oxidant/mole at 5, 15, and 30 minutes, respectively, and yielded 1.0 mole of pentose/mole. Hydrolysis of the oxidation product with 0.33 N sulphuric acid at 100° C for 1 hour yielded arabinose, glucose, cellobiose, and cellotriose as well as two pentose-containing oligosaccharides (detected chromatographically).

2-O- β -D-Cellotriosyl-glyceritol (XIII)

Tetrasaccharide XIV (157 mg) was degraded as described above for the conversion of X to XI. The procedure involved, successively, oxidation with 1 molar proportion of lead tetraacetate, reduction with sodium borohydride, oxidation with 2 molar proportions of lead tetraacetate, and reduction with sodium borohydride. The final product was crystallized from aqueous ethanol (weight, 122 mg), m.p. 234–237° C; the X-ray powder diagram was indistinguishable from that of 2-O- β -D-cellotriosyl-glyceritol* (27), $[\alpha]_D -12.4^\circ$ (c , 1.4, H_2O). Calculated for $C_{21}H_{38}O_{18}$: C, 43.60%; H, 6.62%. Found: C, 43.16%; H, 6.87% (hygroscopic material).

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*Prepared from cellotetraose by a sequence of reactions involving two oxidations with lead tetraacetate and two reductions with sodium borohydride, the procedure being the same as used in degrading cellobiose (XV) to 2-O- β -D-glucopyranosyl-glyceritol (14), and cellotriose to 2-O- β -D-cellobiosyl-glyceritol (see above).